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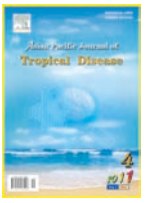

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The antioxidant status and lipid peroxidation product of newly diagnosed and 6 weeks follow-up patients with pulmonary tuberculosis in Owerri, Imo state, Nigeria

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ABSTRACT

Objective: To determine the levels of antioxidants vitamins C and E as well as lipid peroxidation product malondialdehyde (MDA) in *Mycobacterium tuberculosis* (*M. tuberculosis*) patients. **Methods:** Sixty two *M. tuberculosis* positive patients and fifty five healthy controls within the age of twenty five to forty years without any systemic disease attending General Hospital Owerri were involved in this study. Forty one cases were longitudinally followed up with standard anti-tuberculosis chemotherapy (ATT) for six weeks. **Results:** The results obtained showed that the levels of vitamin C (0.91±0.42 mg/dL) and vitamin E (0.84±0.31 mg/dL) were significantly decreased in *M. tuberculosis* patients before treatment when compared to the healthy controls [(1.64±0.41 mg/dL) and (1.46±0.38 mg/dL)] respectively at $P<0.05$, while the level of MDA (8.7±1.81 nM/mL) in *M. tuberculosis* patients was significantly higher ($P<0.05$) before anti-tuberculosis treatment as compared with the healthy control (4.91±1.9 nM/mL). Also, there was a significant increase in vitamin C and E levels after 6 weeks of ATT, while MDA levels was decreased when compared with the control ($P<0.05$). **Conclusions:** The depletion of vitamins C and E as well as elevation of MDA in tuberculosis patients is suggestive of lipid peroxidation and oxidative stress. The increase in vitamin C and E as well as decrease in MDA after 6 weeks of anti-tuberculosis treatment is suggestive of good response to treatment with standard ATT. Hence, vitamin C and E supplementation improves the quality of life of tuberculosis patients.

1. Introduction

Pulmonary tuberculosis is a disease associated with a wide range of respiratory symptoms[1]. It is infectious and contagious disease which apparently develops under conditions of a deficient immunological response[2]. According to the World Health Organization tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) which commonly affects the lungs and is characterized by productive cough, night sweats, fever, chills, chest pain, weightloss, fatigue and anorexia[3]. Other factors that contribute to the resurgence of tuberculosis in developing countries include malnutrition, over crowding, poverty, inadequate treatment and increasing number of displaced persons[4]. Nearly one third of the global population is infected with *M. tuberculosis*[5]. It is estimated that 8.5 million people develop active tuberculosis disease each year resulting in approximately 2 million deaths[1].

The increased production of reactive oxygen species (ROS) is a consequence of phagocyte respiratory burst during *M. tuberculosis* infection. These products of activation are cytotoxic and need to be removed by efficient antioxidant system[6]. Free radicals are thought to play a major role in etiology of a wide variety of disease including *M. tubercosis*. The generation of lipid peroxides indicates the extent of lipid peroxidation and serves as marker of cellular damage[7]. Human tissues are protected from oxidative damage by variety of mechanisms including antioxidants like vitamin C and E[8]. Vitamins play an essential role in the antioxidant defense system[9]. Vitamin C is an important antioxidant that protects the cells against lipid peroxidation as it scavenges superoxide anion, hydrogen peroxide and thiol radicals[10]. Vitamin E is a fat soluble antioxidant that

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can convert superoxide, hydrogen peroxide and lipid peroxyl radicals to less reactive forms[11].

In this study, the antioxidants: vitamin C and E as well as malondialdehyde (MDA) levels were evaluated in newly diagnosed tuberculosis patients as well as 6 weeks after standard antituberculosis chemotherapy (ATT).

2. Materials and methods

2.1. Subjects

Sixty-two patients diagnosed by Ziehl Neelson staining of being positive to *M. tuberculosis* attending the Out-patient Department, General Hospital Owerri participated in the study. Fifty-five healthy controls without any systemic disease, and forty one follow-up patients on standard chemotherapy with isoniazid 5 mg/kg/day, ethanbutal 15 mg/kg/day, and rifampicin 10 mg/kg/day for 6 weeks were also involved. Informed consent of the participants was obtained and was conducted in line with the ethical approval of the hospital.

2.2. Blood Sample

In all subjects, 5 mL of venous blood was collected into EDTA bottles.The plasma samples were obtained by centrifuging the whole blood in Wisterfuge (model 684) at 2 500 g for 10 min and were used for the estimation of plasma vitamins C and E. Plasma vitamin C was assayed by the 2, 4-dinitrophenylhydrazine method described by Tietz[12]. The vitamin E was done by the method of Tietz[13], in which vitamin E caused the reduction of ferric to ferrous ion which then forms a red complex with α-dipyridyl. Lipid peroxidation in plasma was estimated colorimetrically measuring MDA by the method of Nwanjo and Ojiakor[14]. In brief, 0.1 mL of plasma was treated with 2.0 mL of (1:1:1 ratio) TBA-TCA-HCL reagent (TBA 0.37%; HCL 0.25%; TCA 15%) and was placed in water for 5 min followed by cooling and centrifuging. And then clear supernatant was measured at 535 nm against reference blank.

Erythrocyte sedimentation rate (ESR), packed cell volume (PCV) and haemoglobin were estimated by the standard method[4].

2.3. Statistical analysis

The results were expressed as Mean ± SD and students *t*-tests was used to calculate the significance at $P < 0.05$.

3. Results

It was observed that the levels of vitamin C and vitamin E in tuberculosis patients are significantly lower than the healthy control, while the MDA level is higher than the healthy. After 6 weeks follow-up treatment with ATT in tuberculosis patients, the levels of vitamin C and E were improved and the MDA level decreased markedly (Table 1).

The valuse of ESR, hemoglobin and PCV in the healthy and tuberculosis patients were shown in Table 2.

Table 1
The serum levels of vitamin C, vitamin E, and MDA in healthy individuals, pre-treatment and post-treatment tuberculosis patients at follow-up after standard chemotherapy for 6 weeks.

Parameters	Control	Pre-treatment	Post-treatment
Vitamin C (mg/dL)	1.64±0.41	0.91±0.42	1.16±0.44
Vitamin E (mg/dL)	1.46±0.38	0.84±0.31	0.99±0.39
MDA (mol/mL)	4.91±1.90	8.70±1.81	5.27±1.34

Table 2
The values of ESR, hemoglobin and PCV in healthy individuals, pre-treatment, and post-treatment tuberculosis patients at follow up.

Parameters	Control	Pre-treatment	Post-treatment
ESR (mm/hr)	8.30±3.11	126.00±12.4	69.20±7.80
hemoglobin (g/dL)	13.60±2.19	9.40±3.10	11.90±2.60
PCV (%)	39.80±5.63	29.6±4.21	33.90±2.90

4. Discussion

Pulmonary tuberculosis is one of the most common causes of morbidity and mortality in developing nations like Nigeria. It is economically and socially cumbersome to control.

In this present study, it was observed that the antioxidants vitamins C and E were significantly depleted in tuberculosis patients when compared with healthy individuals.

This is in line with the work of Madhab *et al*[1] and Akiibinu *et al*[15]. The lower levels of vitamin C and E levels were associated with excessive ROS production and oxidative stress in tuberculosis[16]. However, after weeks of treatment, the vitamin C and E increased. Vitamin C is the most aqueous phase chain breaking antioxidants which directly scavenges radicals present in the aqueous compartment. While vitamin E, the most important lipid phase chain breaking antioxidants, scavenges radicals in membranes and lipoprotein particles and are central to the prevention of lipid peroxidation[17]. Both vitamin C and E play a protective role against oxidative membrane attack[9].

Indeed free radicals released from *M. tuberculosis* patient initiate lipid peroxidation by attacking polyunsaturated fatty acids in cell membranes, converting them to lipid peroxides and to a variety of secondary metabolites[18]. The uncontrolled peroxidation alters membrane fluidity and permeability. Hence, the lipid peroxides and their secondary metabolites such as MDA are then transported through the circulation by lipoproteins causing damage at distant tissues[17]. In this study, it was observed that there was a higher level of MDA in the *M. tuberculosis* patients than the healthy individuals. However, the level of MDA significantly decreased after 6 weeks of treatment and there is a significant decrease in ROS generation. Hence, the extent of lipid peroxidation is decreased by chemotherapeutic destruction of mycobacteria. This is in line with the work of Reddy *et al*[19]. Madhab *et al*[1] reported that the concentration of MDA level is significantly higher in tuberculosis[20-26]. High MDA production is linked with increased production of ROS and also a marker of the extent of oxidative stress elicited by the immune system. Guler *et*

al[27] and Turgut *et al*[28] reported that tuberculosis stimulates the cellular activation which decreased when treatment was effective. The chemotherapy decrease the MDA level. Furthermore, the levels of haemoglobin, and PCV in tuberculosis patients were significantly reduced, while ESR was increased. Madebo *et al*[29] and Muller *et al*[30] reported that the reduced antioxidants with undernourishment enhance the generation of ROS resulting in increased utilization of these compound which may cause markedly enhanced oxidative stress in tuberculosis. However, with standard chemotheraphy, parameters like haemoglobin and PCV were increased while ESR was decreased significantly ($P<0.05$).

In conclusion, this study suggest that levels of vitamin C and E were decreased while MDA was increased in tuberculosis patients. However, within 6 weeks intensive anti-tuberculosis therapy, the levels of vitamin C and E were increased while MDA was decreased in tuberculosis patients. This warrants the use of antioxidants vitamins as a supplementation in the therapeutic treatment against tuberculosis.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Madhab L, Narayan G, Narendra B, Bishamber D, Shymal K, Nirmal B. Evaluation of lipid peroxidation product, nitrite and antioxidant level in newly diagnosed and two months follow-up patients with pulmonary tuberculosis. *South East Asian J Trop Med Public health* 2007; **4**(38): 695-703.

[2] Madhavi M, Samudruam P, Hemanth A, Lalitha V. Effect of antioxidant vitamins C and E supplementation its plasma levels and on lipid profile in pulmonary tuberculosis patients. *Am J Infect Dis* 2009; **5**(3): 263-272.

[3] World Health Organization. *Global tuberculosis control report. Annex profile of high burden countries.* Geneva, Switzerland: WHO; 2006.

[4] Cheesbrough M. Protein. In: *District laboratory practice in tropical countries*. United Kingdom: Cambridge University Press; 2004, p. 434-436.

[5] World Health Organization. *Tuberculosis cases detection, treatment and monitoring.* Geneva Switzerland: WHO; 2003.

[6] Akiibinu MO, Arinola OG, Ogunyemi EO. Plasma neopterin and peroxide levels in pulmonary tuberculosis patients on chemotherpy with or without micronutrient supplementation. *Pak J Med Sci* 2009; **25**(3): 380-385.

[7] Guteridge JMC. Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin Chem* 1995; **41**: 1819-1828.

[8] Rock CL, Jacob RA, Bowen PE. Update on biological characteristics of the antioxidant micronutrient, vitamins C and E and the caroternoids. *J Am Diet Assoc* 1996; **96**: 69-704.

[9] Debes R, Pradeep D, Kalyan G, Neelam G. Antioxidant vitamin level in sickle cell disorders. *Natl Med J India* 2007; **20**(1): 11-13.

[10] Vijayamalini M, Manoharan S. Lipid peroxidation, vitamin C, E and reduced glutathione level in patients with pulmonary tuberculosis. *Cell Biochem Funct* 2004; **22**: 19-22.

[11] Suhail M, Faizul-suhail M, Khan H. Role of vitamin C and E in regulating antioxidant and prooxidant markers in preclampsia. *J Clin Biochem Nutr* 2008; **43**(3): 210-220.

[12] Tietz W. Ascorbic acid. In: *Fundamental of clinical chemistry.* Britian: W.B. Saunder Company Britain. 1976, p. 422-429.

[13] Tietz W. Clinical guide to laboratory tests. Philadelphia: W.B. Saunders Company; 1976.

[14] Nwanjo HU, Ojiako OA. Effect of vitamin E and C on exercise induced oxidative stress. *Global J Pure Applied Sci* 2005; **12**: 199-202.

[15] Akiibunu MO, Arinola OG, OGunlewe JO, Onih EA. Non enzymatic antioxidant status and nutritional profile in newly diagnosed pulmonary tuberculosis patients in nigeria. *Afr J Biomed Res* 2007; **10**: 223-228.

[16] Arinola OG, Akibinu MO. Plasma complement factor I (CI) and essential trace elements in children with protein energy mal nutrition in ibadan Nigeria. *Trop J Health Sci* 2008; **15**(2): 16-19.

[17] Mohd S, Safia S, Bharat K, Vinay B. Malondialdehyde and antioxidant enzymes in material and cord blood, and their correlation in nornotensive and preclamptic women. *J Clin Med Res* 2009; **1**(3): 150-157.

[18] Jack CL, Jackson MJ, Hind CR. Circulating markers of free radical activity in patients with pulmonary tuberculosis. *Tuber Lung Dis* 1994; **75**: 132-137.

[19] Reddy YN, Murthy SV, Krishna DR, Prabhakar MC. Role of free radicals and antioxidant in tuberculosis patients. *India J Tuberc* 2004; **51**: 213-218.

[20] Immanuel C, Victor L, Chelvi KS, Padmapriyadarsini C, Rehman F, Iliayas S, et al. Serum neoptein levels in HIV infected patients with or without tuberculosis Indian. *Indian J Med Res* 2005; **121**: 220-225.

[21] Bandyopadhyay M, Bhakta A, Chakrabarty S, Pal M, Bharati P. Clinical and bacteriological correlates of whole blood interferon gamma (IFN- γ) in newly detected cases of pulmonary TB. *Asian Pac J Trop Med* 2010; **3**(3): 224-231.

[22] Q Wu, P Zhou, S Qian, X Qin, Z Fan, Q Fu, et al. Cloning, expression, identification and bioinformatics analysis of Rv3265c gene from *Mycobacterium tuberculosis* in *Escherichia coli*. *Asian Pac J Trop Med* 2011; **4**(4): 266-270.

[23] Tiwari N, Kandpal V, Tewari A, K Ram Mohan Rao, Tolia VS. Investigation of tuberculosis clusters in Dehradun city of India. *Asian Pac J Trop Med* 2010; **3**(6): 486-490.

[24] BuijtelsPatricia CAM, Iseman MD, Parkinson S, Cas S de Graaff, Verbrugh HA, Petit PLC, Dick van Soolingen. Misdiagnosis of tuberculosis and the clinical relevance of non-tuberculous mycobacteria in Zambia. *Asian Pac J Trop Med* 2010; **3**(5): 386-391.

[25] Garberi J, Labrador J, Garberi F, Garberi JE, Peneipil J, Garberi M, et al. Diagnosis of *Mycobacterium tuberculosis* using molecular biology technology. *Asian Pac J Trop Biomed* 2011; **1**(2): 89-93.

[26] Singhal S, Jaiswa P. Presentation of tuberculosis in TB-HIV co-infection patients and the treatment outcomewith directly observed short course therapy. *Asian Pac J Trop Biomed* 2011; 1 Suppl 2: S266-267.

[27] Guler M, Huddam D, Unsal E, Ciftci B, Bukan N, Erdogan V, et al. The role serum neoperin level in the evalution of activation and response to treatment in the patients with pulmonary tuberculosis. *Tuberk Toraks* 2006; **54**(4): 330-335.

[28] Turgut T, Akubulut H, Devesi F, Kacar C, Muz MH. Serum interleukin -2- and neopterin level as useful markers for treatment of active pulmonary tuberculosis. *Tohoku J Exp Med* 2006; **209**(4): 321-328.

[29] Madebo T, Lindtjorn B, Aukrust P, Berge RK. Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia. *Am J Clin Nut* 2003; **78**: 117-122.

[30] Muller F, Svardal AM, Nordoy I, Berge RK, Aukrust P, Froland SS. Virological and immunological effects of antioxidant treatment in patients with HIV infection. *Eur J Clin Invest* 2000; **30**: 905-914.